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Localization of a novel autosomal recessive nonsyndromic hearing impairment locus *DFNB65* to chromosome 20q13.2–q13.32

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Abstract

Abstract Autosomal recessive nonsyndromic hearing impairment (ARNSHI) is the most frequent form of prelingual hereditary hearing loss in humans. Between 75 and 80% of all nonsyndromic deafness is inherited in an autosomal recessive pattern. Using linkage analysis, we have mapped a novel gene responsible for this form of nonsyndromic hearing impairment, *DFNB65*, in a consanguineous family from the Azad Jammu and Kashmir regions, which border Pakistan and India. A maximum multipoint LOD score of 3.3 was obtained at marker D20S840. The three-unit support interval is contained between markers D20S902 and D20S430, while the region of homozygosity is flanked by markers D20S480 and D20S430. The novel locus maps to a 10.5-cM region on chromosome 20q13.2–q13.32 and corresponds to a physical map distance of 4.3 Mb. *DFNB65* represents the first ARNSHI locus to map to chromosome 20.

Keywords

Autosomal recessive nonsyndromic hearing impairment; *DFNB65*; Pakistan; 20q13.2–q13.32

Introduction

It is estimated that 1–2 per 1,000 newborns are affected with profound hearing impairment (HI) [1]. For congenital HI, roughly half of the cases are expected to be genetic. Of the genetic cases of HI, 30% are known to be syndromic, with the remaining cases displaying nonsyndromic hearing impairment (NSHI). For NSHI, 70–75% of the cases are due to autosomal recessive (AR) inheritance, 15–20% display autosomal dominant inheritance, 2–3% of cases show X-linked inheritance, and less than 1% of cases are due to mitochondrial inheritance [2]. Thus far, for ARNSHI more than 60 loci have been mapped and 21 genes identified [3]. This extreme heterogeneity reflects involvement of different molecular mechanisms within the auditory system that malfunction to cause HI. In the current study, we investigated a consanguineous family segregating ARNSHI and mapped the gene responsible for the defect to chromosome 20q13.2–q13.32.

Materials and methods

Family history

Before the start of the study, approval was obtained from the Quaid-I-Azam University and the Baylor College of Medicine Institutional Review Boards. Informed consent was obtained from all family members who agreed to participate in the study. Family 4020 (Fig. 1), which is from the city of Rawlakot in Azad Jammu and Kashmir region that borders Pakistan and India, provided convincing evidence of autosomal recessive mode of inheritance. All affected individuals have a history of prelingual profound HI and use sign language for communication. In addition, all available medical records of the affected individuals were scrutinized for evidence of childhood illness. Each HI individual underwent a physical and ophthalmologic examination. Defects in ear morphology, dysmorphic facial features, eye disorders including night blindness and tunnel vision, limb deformities, mental retardation, and other clinical features that could indicate that HI was syndromic were not identified. The clinical history and physical examination of affected individuals indicated no vestibular involvement.

Figure 2 exhibits the audiometric profile of hearing-impaired individuals IV-2 and IV-3, aged 17 and 15 respectively, who have profound HI at all frequencies in the two ears. The audiograms also display a flat configuration.

Extraction of genomic DNA and genotyping

Peripheral blood samples were obtained from six family members, four of whom were hearing-impaired. Genomic DNA was extracted from whole blood following a standard protocol [4], quantified by spectrophotometric reading at optical density 260, and diluted to 40 ng/μl for polymerase chain reaction (PCR) amplification. A genome scan was carried out on six DNA samples at the Center for Inherited Disease Research (CIDR). A total of 405 fluorescently labeled short tandem repeat markers were genotyped. These markers are spaced at approximately 10 cM apart and are located on 22 autosomes and X and Y chromosomes. After the completion of the genome scan, two additional unaffected (IV-1 and IV-5) family members were ascertained and their DNA samples were used for fine mapping of the *DFNB65* locus.

For fine mapping, microsatellite markers were amplified by PCR according to standard procedure in a total volume of 25 μl with 40 ng genomic DNA, 0.3 μl of primer (Invitrogen Corp., Carlsbad, CA, USA), 200 μM deoxyribonucleotide triphosphate and 1 unit of Taq DNA Polymerase (Fermentas Life Sciences, Burlington, ON, Canada) in a thermal cycler (Whatman Biometra, Goettingen, Germany). PCR products were resolved on 8% nondenaturing polyacrylamide gel.

Linkage analysis

The National Center for Biotechnology Information Build 34 sequence-based physical map was used to determine the order of the genome scan markers and fine mapping markers [5]. Genetic map distances according to the Rutgers combined linkage-physical map of the human genome [6] were used to carry out the multipoint linkage analysis for the fine map and genome scan markers. For those genome scan markers where no genetic map position was available, interpolation was performed to place these markers on the Rutgers combined linkage-physical map. PEDCHECK [7] was used to identify Mendelian inconsistencies, while the MERLIN [8] program was utilized to detect potential genotyping errors that did not produce a Mendelian inconsistency. Haplotypes were constructed using SIMWALK2 [9,10]. Two-point linkage analysis was carried out using the MLINK program of the FASTLINK computer package [11], and multipoint linkage analysis was performed using ALLEGRO [12]. An autosomal recessive mode of inheritance with complete penetrance and a disease allele frequency of 0.001 were assumed. Marker allele frequencies were estimated from the founders and the reconstructed genotypes of founders from this family and 32 additional Pakistani families that underwent a genome scan at the same time at CIDR. Equal allele frequencies were used for fine mapping markers because it was not possible to estimate allele frequencies from the founders, because these markers were only genotyped in this family. Because false positive results can be obtained when analyzing the data using too low of an allele frequency for the allele segregating with the disease locus [13], a sensitivity analysis was carried out for the multipoint linkage analysis by varying the allele frequency of the allele that is segregating with the disease locus from 0.2 to 0.7 for the fine mapping markers.

Results

Two point linkage analysis of the genome scan markers generated maximum LOD scores at $\theta=0$ of 1.2 and 1.9 with markers D20S480 and D20S451, respectively. A maximum multipoint LOD score of 1.4 was obtained at D20S451. Figure 3 is a genome-wide representation of the multipoint LOD scores for the 22 autosomes. Only on chromosome 20 was a LOD score greater than 1.0 achieved.

To fine-map this region on chromosome 20, 33 additional markers located in the vicinity of D20S480 and D20S451 were selected from the Marshfield genetic map [14]. Sixteen of the markers were uninformative, although all markers that were selected had a reported heterozygosity of >0.7 . Of the 17 fine mapping markers that are informative for linkage, eight markers (including D20S428, D20S877, D20S606, and D20S902) lie between genome scan markers D20S481 and D20S480, while seven markers (D20S840, D20S876, D20S1085, D20S832, D20S100, D20S102, and D20S430) are between D20S480 and genome scan marker D20S451. In addition, D20S164 and D20S171, two of the fine mapping markers, are distal to the genome scan marker D20S451. Analysis of the genome scan and fine mapping marker genotypes within this region with PEDCHECK and MERLIN did not elucidate any genotyping errors. Table 1 summarizes the two-point LOD scores obtained after fine mapping. The maximum two-point LOD score of 2.4 was obtained with five markers (D20S840, D20S1085, D20S832, D20S100 and D20S102) at $\theta=0$. Multipoint linkage analysis resulted in a maximum LOD score of 3.3 at marker D20S840 (Fig. 4). The three-unit support interval was between markers D20S902 and D20S430, spanning 11.7 cM according to the Rutgers combined linkage-physical map of the human genome [6] and corresponding to a physical map distance of 4.4 Mb (Table 1).

Haplotypes were constructed to determine the critical recombination events in the family (Fig. 1). All hearing-impaired individuals were homozygous for markers D20S902 and D20S480, but unaffected individual IV-1 is also homozygous for the same markers. Thus,

the centromeric boundary of the interval is assigned between markers D20S480 and D20S840. The telomeric boundary of the interval corresponds to a recombination event between markers D20S102 and D20S430, which occurred in unaffected individual IV-1. The region of homozygosity that is flanked by markers D20S480 and D20S430 contains 4.3 Mb and is 10.5-cM long according to the Rutgers combined linkage-physical map of the human genome (Table 1). The region of homozygosity is therefore narrower than the three-unit support interval and most likely includes the gene for *DFNB65*.

When the marker allele frequencies for the alleles segregating with HI were varied for the fine mapping markers between 0.2 and 0.4, the maximum multipoint LOD score remained to be 3.3 at marker D20S840. When the allele frequencies were increased, the maximum LOD score still occurred at marker D20S840 but decreased to 3.2 and 3.1 at allele frequencies of 0.6 and 0.7, respectively.

Discussion

The linkage data presented here suggest that a novel gene for ARNSHI is located on chromosome 20q13.2–q13.32. This is the first HI locus identified on human chromosome 20. Many of the known nonsyndromic HI loci have been identified in single large families in which linkage can be established independently. However, due to the limited number of meioses within each family, the genetic interval for the HI locus is often large, which makes gene identification difficult.

The *DFNB65* interval spans 4.3 Mb and contains 17 known genes (Table 2). Of these, bone morphogenetic protein 7 (*BMP7*, MIM 112267) has extensive expression in the otic placode of embryonic chick, which eventually becomes restricted to cochlear supporting cells over time [18]. This makes the *BMP7* gene the best candidate of the known genes in the *DFNB65* locus. However, sequencing of DNA for the *BMP7* gene from one unaffected and two hearing-impaired individuals from family 4020 resulted in no functional sequence variants within the promoter and exonic regions.

Other genes within the interval that are included in expression databases for the inner ear [19,20] are breast carcinoma amplified sequence 1 (*BCAS1*, MIM 602967) and prefoldin 4 (*PFDN4*, MIM 604898). These two genes have been implicated in several soft tissue cancers (e.g., breast, prostate, colon), which may indicate the oncogenic potential of these genes, although their specific functions remain unknown.

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Biography

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SUZANNE M. LEAL received her M.S. in biostatistics and Ph.D. in epidemiology from Columbia University in New York City, USA. She is currently an Associate Professor in the Department of Molecular and Human Genetics at Baylor College of Medicine. Her research interests include gene mapping of complex and Mendelian traits, the genetics of nonsyndromic hearing impairment, and the study of methodological problems in statistical genetics.

Abbreviations

HI	hearing impairment
NSHI	nonsyndromic HI
ARNSHI	autosomal recessive NSHI

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Fig. 1. Pedigree drawing of family 4020. *Black symbols* represent individuals with hearing impairment. *Clear symbols* represent unaffected individuals. The gender of some of the family members was changed to protect the anonymity of the family. Haplotypes are shown below each individual for whom genotypes are available. For individuals in generation IV, maternal haplotypes are displayed on the *left-hand side* and paternal haplotypes on the *right-hand side*. *Shaded areas* indicate the region of homozygosity

Fig. 2.

Audiograms of hearing-impaired individuals **a** IV-2 and **b** IV-3 of family 4020, demonstrating profound hearing impairment involving all frequencies in the two ears. The normal audiograms of family members **c** III-2, **d** IV-1 and **e** IV-5 are also shown. Individual IV-5 is aged 21 years, while IV-2 is 17 and IV-3 15 years of age. *Circles and crosses* represent air conduction for right and left ear, respectively. *Triangles* represent masked air conduction thresholds

Fig. 3.

Genome-wide plot of multipoint LOD scores from the genome scan genotypes of family 4020. The LOD score is plotted against the genetic map distance in centimorgans (cM) per chromosome. Only on chromosome 20 at marker D20S451 was a multipoint LOD score of more than 1.0 (LOD=1.4) derived

Fig. 4.
The plot of the multipoint LOD scores including the fine mapping markers on chromosome 20 (*broken line*) is superimposed on the LOD score plot from the genome scan markers (*solid line*)

Table 1

Two-point LOD score results between the *DFNB65* locus and chromosome 20 markers after fine mapping

Marker ^a	Genetic map position ^b	Physical map position ^c	LOD score at $\theta=$									
			0.0	0.01	0.02	0.04	0.05	0.1	0.2	0.3		
D20S428	80.34	51,588,497	– ∞	–1.39	–0.83	–0.32	–0.17	0.20	0.34	0.24		
D20S877	80.49	51,799,558	0.85	0.83	0.81	0.78	0.76	0.67	0.48	0.29		
D20S606	81.41	52,247,558	GO	0.31	0.56	0.77	0.81	0.88	0.70	0.41		
D20S902	81.41	52,436,690	1.15	1.13	1.10	1.05	1.03	0.91	0.66	0.41		
D20S480^d	82.62	52,542,629	1.31	1.28	1.26	1.20	1.17	1.03	0.75	0.46		
D20S840	82.80	52,832,756	2.35	2.30	2.25	2.15	2.09	1.83	1.29	0.75		
D20S876	83.13	53,033,103	1.15	1.13	1.10	1.05	1.03	0.91	0.66	0.41		
D20S1085	84.67	53,366,902	2.35	2.30	2.25	2.15	2.09	1.83	1.29	0.75		
D20S832	86.80	54,580,765	2.35	2.30	2.25	2.15	2.09	1.83	1.29	0.75		
D20S100	88.19	54,999,475	2.35	2.30	2.25	2.15	2.09	1.83	1.29	0.75		
D20S102	89.21	55,676,797	2.35	2.30	2.25	2.15	2.09	1.83	1.29	0.75		
D20S430	93.11	56,835,225	– ∞	0.61	0.86	1.06	1.10	1.15	0.93	0.57		
<i>D20S451^d</i>	<i>94.79</i>	<i>57,349,950</i>	– ∞	<i>0.81</i>	<i>1.06</i>	<i>1.25</i>	<i>1.29</i>	<i>1.31</i>	<i>1.04</i>	<i>0.63</i>		
D20S164	95.96	57,738,776	–1.17	–0.49	–0.28	–0.08	–0.02	0.07	0.02	–0.03		
D20S171	98.00	58,493,299	– ∞	–2.19	–1.66	–1.15	–1.00	–0.56	–0.23	–0.09		

Therefore, the results reported in this table for the genome scan markers are different from those obtained from the initial genome scan

^aMarkers in bold type flank the haplotype. Genome scan markers are shown in italics

^bCumulative sex-averaged Kosambi cM genetic map distances from the Rutgers combined linkage-physical map of the human genome [6]

^cSequence-based physical map distances in bases according to Build 34 of the human reference sequence [5]

^dThe two unaffected individuals who were not included in the genome scan were also genotyped for these genome scan markers.

Table 2

DFNB65 candidate genes

Gene	Protein	MIM	Physical map position	Cytogenetic band	Description
<i>ZNF217</i>	Zinc finger protein 217	602967	52,869,034	20q13.2	Putative oncogene
<i>BCAS1</i>	Breast carcinoma amplified sequence 1	602968	53,245,939	20q13.2	Putative oncogene
<i>CYP24A1^a</i>	Cytochrome P450, family 24 precursor	126065	53,455,410	20q13.2	Has role in calcium metabolism and blood cell differentiation
<i>PFDN4</i>	Prefoldin 4	604898	53,509,924	20q13.2	Molecular chaperone; putative oncogene
<i>DOK5</i>	DOK5 protein isoform b	608334	53,777,679	10q13.2	Docking protein
<i>CBLN4</i>	Cerebellin 4 precursor	–	55,257,918	20q13.31	Neuromodulatory glycoprotein in adrenal medulla
<i>MC3R^b</i>	Melanocortin 3 receptor	155540	55,509,211	20q13.31	Associated with obesity
<i>STK6</i>	Serine/threonine protein kinase 6	602687	55,629,867	20q13.31	Has role in spindle formation and chromosomal segregation
<i>CSTF1</i>	Cleavage stimulation factor sub-unit 1	600369	55,653,041	20q13.31	Ensures correct polyadenylation of mRNA
<i>TFAP2C^c</i>	Transcription factor AP-2 gamma	601602	55,889,780	20q13.31	Regulator of gene expression for ectodermal tissue development
<i>BMP7</i>	Bone morphogenetic protein 7 precursor	112267	56,430,977	20q13.31	Member of transforming growth factor-beta superfamily
<i>SPO11</i>	Meiotic recombination protein SPO11 isoform a	605114	56,590,253	20q13.31–q13.32	Required for meiotic double strand break formation
<i>RAE1</i>	RAE1 (RNA export 1, S.pombe) homolog	603343	56,611,567	20q13.32	May function in nucleocyto-plasmic transport and attaching cytoplasmic mRNA-binding protein to cytoskeleton
<i>RNPC1</i>	RNA-binding region containing protein 1 isoform	–	56,651,885	20q13.32	Probable RNA-binding protein
<i>HMG1L1</i>	High-mobility group (nonhistone chromosomal)	–	56,748,868	20q13.32	May be involved in tumorigenesis
<i>CTCF</i>	CCCTC-binding factor-like protein	607022	56,757,645	20q13.32	Putative oncogene
<i>PCK1</i>	Cytosolic phosphoenolpyruvate carboxykinase 1	261680	56,821,558	20q13.32	Main target for regulation of gluconeogenesis

This list does not include five genes that encode hypothetical proteins: *C20orf108* (*LOC116151*), *C20orf32* (*HEF-like protein*), *C20orf43* (*LOC51507*), *C20orf106* (*LOC200232*) and *LOC388799*

^aThe *Cyp24a1* rat manifests with albuminuria and hyperlipidemia [15]

^b*Mc3r*– mice have increased fat mass [16]

^cThe *Tjp2c* knockout is embryonic lethal in mice [17]